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Gene Networks Underlying Chronic Sleep Deprivation in Drosophila

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14. ABSTRACT
Studies of the gene network affected by sleep deprivation and stress in the fruit fly Drosophila have revealed the involvement of a variety of genes known for their role in neural development and function. In particular, the TGF-alpha/EGF-receptor and Wnt/Frizzled signal transduction pathways are affected. Subseuqent tests of mutants in these pathways demonstrated a strong effect on sleep maintenance. Further investigation of genes directly affected by alterations in these two pathways revealed a set of genes already known for their putative role in autism, a syndrome known for its pronounced deficits in sleep, its elevated stress response, and its cognitive defects. These

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Report Title

Gene Networks Underlying Chronic Sleep Deprivation in Drosophila

ABSTRACT

Studies of the gene network affected by sleep deprivation and stress in the fruit fly Drosophila have revealed the involvement of a variety of genes known for their role in neural development and function. In particular, the TGF-alpha/EGF-receptor and Wnt/Frizzled signal transduction pathways are affected. Subseuqent tests of mutants in these pathways demonstrated a strong effect on sleep maintenance. Further investigation of genes directly affected by alterations in these two pathways revealed a set of genes already known for their putative role in autism, a syndrome known for its pronounced deficits in sleep, its elevated stress response, and its cognitive defects. These findings point to promising leads for future research to establish the causal connections between stress and neural function, and to identify strategies for ameliorating their detrimental effects.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Ralph Greenspan	0.30	
FTE Equivalent:	**0.30**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: .....

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):.....

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:.....

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense .....

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: .....

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Jenee Wagner	0.25
Diana Lam	0.10
Saad Yazdani	0.10
Kim Ha	0.10
Robert Buffington	0.08
FTE Equivalent:	**0.63**
Total Number:	**5**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

We had previously reported, based on gene expression profiling in sleep deprived and stressed flies, the involvement of axonogenesis as a process regulated by these stressors. This goes beyond the current hypothesis of sleep as functioning mainly to restore synaptic homeostasis, to indicate that severe deprivations appear to be triggering regenerative processes in neurons, presumably in response to crisis signals due to the stressors. The involvement of these pathways in adult brain itself is not entirely new, as there is an emerging literature on the role of the Wnt pathway, in particular, in regulating the potential for nerve regeneration in adult mice.

We had subsequently followed up with a demonstration of the role of the TGF-alpha/EGFR signaling pathway in these processes. (Our attempts to publish these results were thwarted by another group that pre-empted us.)

We have subsequently returned to our original gene expression results to test the role of genes implicated from it. Since the Wnt pathway had been implicated, we confirmed its role in sleep maintenance by showing that the Wnt receptor, encoded by the frizzled gene in Drosophila, is required. Specifically, flies with a 50% reduction in frizzled expression level exhibit a significant increase in sleep. (The homozygous fz/fz genotype is lethal.)

To explore the gene network surrounding this phenomenon, we performed further gene expression analyses on fz/+ as compared to the control CS strain. These tests revealed differential expression of a range of synapse-related genes. Many of these are also implicated in autism, which itself has a pronounced effect on sleep, reducing it and increasing its fragmentation, and on stress response, in which affected individuals show an elevated stress response as compared to normal controls. These results have interesting implications for future research directions on this set of genes that are clearly pleiotropic for sleep regulation, stress response, and cognitive function.

Technology Transfer

Gene Networks Underlying Chronic Sleep Deprivation in Drosophila

We had previously reported, based on gene expression profiling in sleep deprived and stressed flies, the involvement of axonogenesis as a process regulated by these stressors. This goes beyond the current hypothesis of sleep as functioning mainly to restore synaptic homeostasis, to indicate that severe deprivations appear to be triggering regenerative processes in neurons, presumably in response to crisis signals due to the stressors. The involvement of these pathways in adult brain itself is not entirely new, as there is an emerging literature on the role of the Wnt pathway, in particular, in regulating the potential for nerve regeneration in adult mice.

We had subsequently followed up with a demonstration of the role of the TGF-alpha/EGFR signaling pathway in these processes. (Our attempts to publish these results were thwarted by another group that pre-empted us.)

We have subsequently returned to our original gene expression results to test the role of genes implicated from it. Since the Wnt pathway had been implicated, we confirmed its role in sleep maintenance by showing that the Wnt receptor, encoded by the *frizzled* gene in Drosophila, is required. Specifically, flies with a 50% reduction in *frizzled* expression level exhibit a significant increase in sleep fragmentation (see Figures 1 and 2, below). (The homozygous *fz/fz* genotype is lethal.)

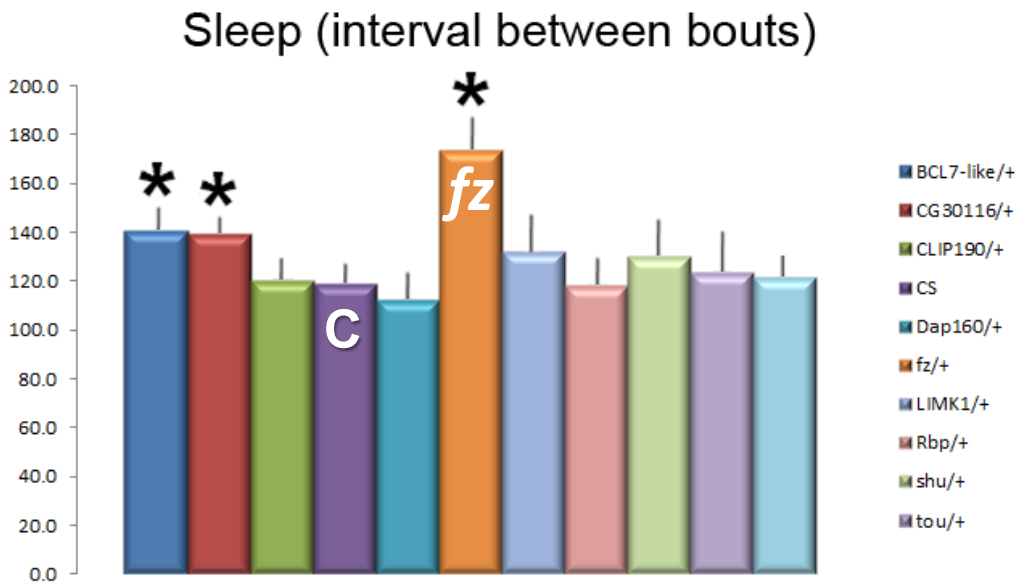

Fig. 1. Bout interval (mean number of minutes between sleep episodes) a measure of sleep fragmentation. (n=16). “C” is the control strain.

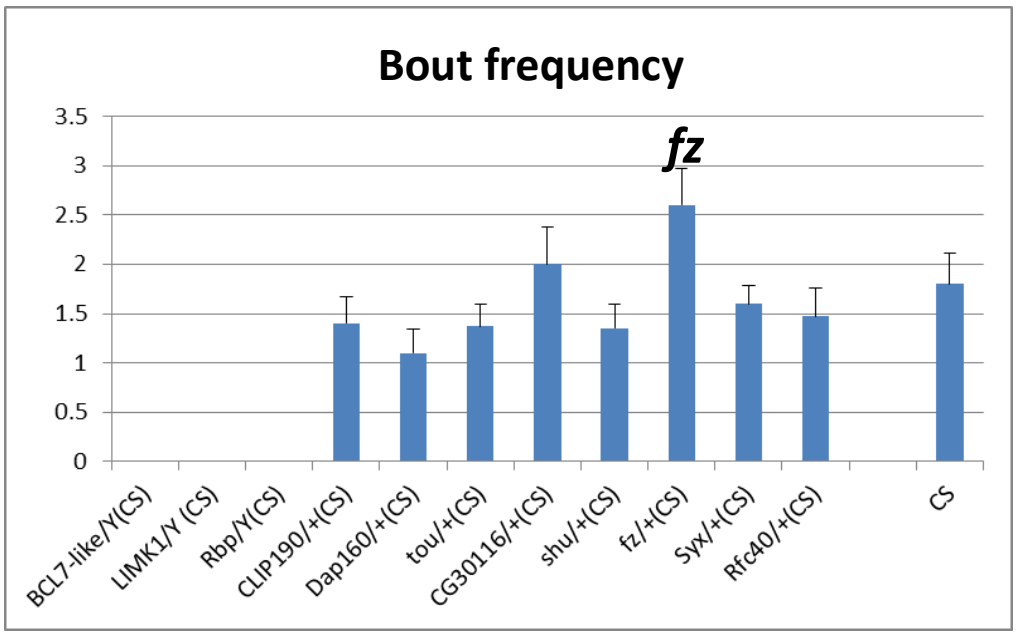

Fig. 2. Bout frequency (mean number of sleep bouts per 30 min period) a measure of sleep fragmentation. (n=16). "C" is the control strain.

To explore the gene network surrounding this phenomenon, we performed further gene expression analyses on *fz/+* as compared to the control CS strain. These tests revealed differential expression of a range of synapse-related genes. Many of these are also implicated in autism, which itself has a pronounced effect on sleep, reducing it and increasing its fragmentation, and on stress response, in which affected individuals show an elevated stress response as compared to normal controls.

RNAseq of fz/+ vs. CS control

Human gene	Fly gene	*fz*/CS
CNTNAP2	neuroligin	1.363
EN2	en	0.776
SHANK3	Prosap	0.770
ITGB3	mys	0.707
ITGB4	β-Int-nu	0.857
MET	Alk	2.342
SLC9A9	Nhe3	0.714
GRIN2B	Nmdar1	0.702
SCN1A,2A	CG9701	1.266

These results have interesting implications for future research directions on this set of genes that are clearly pleiotropic for sleep regulation, stress response, and cognitive function.